

### **AMENDMENTS TO THE CLAIMS**

This listing of claims will replace all prior versions, and listing, of claims in the application.

#### **Listing of Claims:**

Claims 1- 59. (Canceled)

60. (Currently amended) A method for treating a specimen of semen containing sperm cells to increase the relative number of sperm cells of a preferred sex type in a treated specimen to increase the potential for conceiving an offspring of the preferred sex, the method comprising:

collecting a sample of semen, wherein the sample of semen is collected as an ejaculate;

holding the semen after collection for a predetermined period of time;

after the predetermined period of time, separating the semen into two components comprising a first component having a higher number of sperm of the preferred sex type than sperm of a non preferred sex type and a second component having a higher number of sperm of the non preferred sex type relative to sperm of the preferred sex type,

wherein the separating step is performed in a window of time after ejaculation that ~~can be~~ is determined by locating a maximum in a curve obtained by plotting percent female cells determined by FISH against percent Koo positive cells for aliquots of another semen sample, the aliquots being taken at various times after collection, determining the time at which the maximum percent female cells occurs, and determining the period of time for holding the semen after collection before and beginning the separation step to provide a time no earlier than about one hour before the time of the maximum percent female cells as determined by FISH.

61. (Original) The method of claim 60, wherein the separating step comprises contacting the sperm with a cell binding agent; permitting the sperm of the non preferred sex type to preferentially bind to the cell binding agent, and separating the cell binding agent with preferentially bound sperm of the non preferred sex type from non bound sperm, thereby providing said first component.

62. (Original) The method of claim 60, wherein the first component comprises a higher number of X-chromosome bearing sperm relative to Y- chromosome bearing sperm and the second component comprises a higher number of Y- chromosome bearing sperm relative to X- chromosome bearing sperm.

63. (Original) The method of claim 60, further comprising cooling the semen before performing the separating step.

64. (Original) The method of claim 60, further comprising collecting the semen and immediately cooling the semen.

65. (Original) The method of claim 64, wherein the semen is cooled to at least about 16°C.

66. (Original) The method of claim 64, wherein the semen is cooled to at least about 12°C.

67. (Withdrawn) A method for increasing the probability of producing an offspring of a mammalian species having a preferred sex, said method comprising:

collecting a sample of semen;

holding the semen after collection for a predetermined period of time;

after the predetermined period of time, separating semen containing sperm cells of the mammalian species into two components comprising a first component having a higher number of sperm of the preferred sex type than sperm of a non preferred sex type and a second component having a higher number of sperm of the non preferred

sex type relative to sperm of the preferred sex type, wherein the separating step is performed in a window of time that can be determined by locating a maximum in a curve obtained by plotting percent female cells determined by FISH against percent Koo positive cells for aliquots of a semen sample, the aliquots being taken at various times after collection, determining the time at which the maximum percent female cells occurs, and determining the period of time for holding the semen after collection and before beginning the separation step to provide a time no earlier than about one hour before the time of the maximum percent female cells as determined by FISH.

68. (Withdrawn) The method of claim 67, wherein the separating step comprises contacting the sperm with a cell binding agent; permitting the sperm of the non preferred sex type to preferentially bind to the cell binding agent, and separating the cell binding agent with preferentially bound sperm of the non preferred sex type from non bound sperm, thereby providing said first component.

69. (Withdrawn) The method of claim 67, wherein the first component comprises a higher number of X-chromosome bearing sperm relative to Y- chromosome bearing sperm and the second component comprises a higher number of Y- chromosome bearing sperm relative to X- chromosome bearing sperm.

70. (Withdrawn) The method of claim 67, further comprising cooling the semen before performing the separating step.

71. (Withdrawn) The method of claim 67, further comprising collecting the semen and immediately cooling the semen.

72. (Withdrawn) The method of claim 71, wherein the semen is cooled to about 12°C.

73. (Withdrawn) The method of claim 69, wherein the first component contains two-thirds viable X- chromosome bearing sperm and one-third viable Y - chromosome bearing sperm.

74. (Withdrawn) The method of claim 67, wherein twice as many female calves are born than male calves when the treated semen is used to fertilize a plurality of eggs.

75. (Withdrawn) A method for separating a selected population of cells from a sample of semen, the method comprising:

- collecting a sample of semen;
- cooling the semen to a predetermined temperature;
- holding the semen after collection for a predetermined period of time;
- wherein a separation step is performed in a window of time that can be determined by locating a maximum in a curve obtained by plotting percent female cells determined by FISH against percent Koo positive cells for aliquots of another semen sample, the aliquots being taken at various times after collection, determining the time at which the maximum percent female cells occurs, and determining the period of time for holding the semen after collection before beginning the following step to provide a time no earlier than about one hour before the time of the maximum percent female cells as determined by FISH;
- contacting the sample with a cell binding agent to preferentially bind to the selected population of cells for a time sufficient for the cell binding agent to bind the selected cells; and
- separating the selected cells and the cell binding agent to provide a treated sample containing non selected cells.

76. (Withdrawn) The method of claim 75, wherein the treated sample comprises a population of cells wherein a first number of cells exhibiting a preferred sex type is at least about 10% greater than a second number of cells exhibiting a non preferred sex type.

77. (Withdrawn) The method of claim 75, wherein the treated sample comprises a population of cells wherein a first number of cells exhibiting a preferred sex type is at least about 20% greater than a second number of cells exhibiting a non preferred sex type.

78. (Withdrawn) The method of claim 75, wherein the treated sample comprises a population of cells wherein a first number of cells exhibiting a preferred sex type is at least about 50% greater than a second number of cells exhibiting a non preferred sex type.

79. (Withdrawn) The method of claim 75, wherein the treated sample comprises a population of cells wherein a first number of cells exhibiting a preferred sex type is at least about 100% greater than a second number of cells exhibiting a non preferred sex type.

80. (Withdrawn) The method of claim 75, wherein the cell binding agent comprises a bead support having a diameter of 0.1 to 2 microns.

81. (Withdrawn) The method of claim 75, wherein the number of cells in the selected population of cells is greater than about  $1 \times 10^5$  cells/ml.

82. (Withdrawn) A method of insemination, the method comprising obtaining semen containing a population of spermatozoa according to the method of claim 75 and inseminating a mammal with the treated sample containing a population of spermatozoa.

83. (Withdrawn) The method of claim 82, wherein the mammal is selected from the group consisting of cattle, sheep, pigs, goats, horses, dogs and cats.

84. (Withdrawn) The method of claim 82, wherein the semen is cooled to at least about 16°C.

85. (Withdrawn) The method of claim 82, wherein the semen is cooled to at least about 12°C.

86. (Withdrawn) A method of increasing the percentage of mammalian offspring of a predetermined sex, the method comprising:

collecting a specimen of semen;

cooling the specimen of semen containing a population of cells to a predetermined temperature;

incubating the specimen of semen for a predetermined amount of time at the predetermined temperature, wherein the predetermined amount of time can be determined by a method comprising the steps of:

collecting a test specimen of semen,

incubating a first portion of the test specimen at a constant temperature for first incubation period,

incubating a second portion of the test specimen at a constant temperature for a second incubation period,

contacting the first and second portions of the test specimen at the end of the incubation periods with a binding agent which binds a selected population of cells,

separating the first and second portions of the test specimen into selected and non selected test specimens, and

determining which of the incubation time periods yielded the maximum percent female cells in the selected or non selected test specimens, thereby identifying the predetermined amount of time for incubating the semen;

contacting the sample with a cell binding agent to preferentially bind to a selected population of cells for a time sufficient for the cell binding agent to bind the selected cells;

separating the selected cells and the cell binding agent to provide a treated sample containing non selected cells; and

administering at least a portion of the treated sample to the reproductive tract of a female mammal.

87. (Withdrawn) The method of claim 86, wherein the treated sample comprises cells exhibiting a preferred sex type that is at least about 10% greater than the number of cells exhibiting a non preferred sex type.

88. (Withdrawn) The method of claim 86, wherein the treated sample comprises cells exhibiting a preferred sex type that is at least about 20% greater than the number of cells exhibiting a non preferred sex type.

89. (Withdrawn) The method of claim 86, wherein the treated sample comprises cells exhibiting a preferred sex type that is at least about 50% greater than the number of cells exhibiting a non preferred sex type.

90. (Withdrawn) The method of claim 86, wherein the treated sample comprises cells exhibiting a preferred sex type that is at least about 100% greater than the number of cells exhibiting a non preferred sex type.

91. (Withdrawn) The method of claim 86, wherein the cell binding agent comprises a bead support having a diameter of 0.1 to 2 microns.

92. (Withdrawn) The method of claim 86, wherein the number of cells in the selected population of cells is greater than about  $1 \times 10^5$  cells/ml.

93. (Withdrawn) The method of claim 86, wherein the mammal is selected from the group consisting of cattle, sheep, pigs, goats, horses, dogs and cats.

94. (Withdrawn) The method of claim 93, wherein the semen is cooled to at least about 16°C.

95. (Withdrawn) The method of claim 93, wherein the semen is cooled to at least about 12°C.

96. (Withdrawn) The method of claim 75, wherein the time at which the maximum percent of female cells occurs is when at least about 25% of the sperm cells exhibit sticky patches.

96. (Withdrawn) The method of claim 86, wherein the determining step comprises plotting a curve which is obtained by plotting the percent female cells determined by FISH in the non selected test specimen against the percent of H-Y positive cells.

97. (Withdrawn) The method of claim 75, wherein the time at which the maximum percent of female cells occurs is when at least about 30% of the sperm cells exhibit sticky patches.

98. (Withdrawn) The method of claim 75, wherein the time at which the maximum percent of female cells occurs is when at least about 20% but no more than 30% of the sperm cells exhibit sticky patches.

99. (Withdrawn) The method of claim 75, wherein the separating of the selected cells and the cell binding agent step begins 2 hours post collection.

100. (Withdrawn) The method of claim 75, wherein the separating of the selected cells and the cell binding agent step begins 4 hours post collection.

101. (Withdrawn) The method of claim 75, wherein the separating of the selected cells and the cell binding agent step begins 6 hours post collection.

102. (Withdrawn) The method of claim 75, wherein the predetermined temperature is at least 12°C and the separating of the selected cells and the cell binding agent step begins 6 hours post collection.

103. (Withdrawn) A method for determining the optimum time to incubate a sample of semen prior to contacting the sample with a binding agent in order to enrich for female sperm cells, the method comprising:

- (a) collecting a test specimen of semen;
  - (b) cooling the test specimen to a predetermined temperature;
  - (c) incubating the test specimen at the predetermined temperature for a first incubation period;
  - (d) contacting the test specimen with a Koo antibody at the end of the incubation period;
  - (e) separating the test specimen into Koo bound and Koo unbound test specimens;
  - (f) determining the percent of female sperm cells in the Koo unbound test specimen;
  - (g) repeating at least steps (c) through (f) for at least a second incubation period;
- and
- (h) determining the incubation period that resulted in enrichment of the maximum percent of female cells.

104. (Withdrawn) The method of claim 103, wherein step (f) is performed after step (g) and before step (h)

105. (Withdrawn) The method of claim 103, wherein step (h) comprises plotting a curve which is obtained by plotting the percent female cells in the Koo unbound test specimen against the percent of Koo positive cells.

106. (Withdrawn) A method for determining the optimum time to incubate a sample of semen prior to contacting the sample with a binding agent in order to enrich for female sperm cells, the method comprising:

- collecting a test specimen of semen;
- incubating the test specimen at a constant temperature for two or more different incubation periods;

contacting the test specimen at the end of the two or more incubation periods with a binding agent which binds the selected population of cells;

separating the test specimen into selected and non selected test specimens; and

determining which of the two or more incubation time periods yielded the maximum percent female cells in the non selected test specimens.

107. (Withdrawn) The method of claim 106, wherein said determining step comprises plotting a curve which is obtained by plotting the percent female cells against the percent of H-Y positive cells in the separated test semen specimens at the two or more time points.

108. (Withdrawn) The method of claim 106, wherein the binding agent is a H-Y binding agent.

109. (Withdrawn) The method of claim 108, wherein the H-Y binding agent is a Koo antibody and the H-Y positive cells are Koo positive cells.

110. (Withdrawn) The method of claim 106, wherein the separated test semen comprises a population of cells wherein the female sperm cells are at least about 10% greater than the male sperm cells.

111. (Withdrawn) The method of claim 106, wherein the separated test semen comprises a population of cells wherein the female sperm cells are at least about 20% greater than the male sperm cells.

112. (Withdrawn) The method of claim 106, wherein the separated test semen comprises a population of cells wherein the female sperm cells are at least about 50% greater than the male sperm cells.

113. (Withdrawn) The method of claim 106, wherein the separated test semen comprises a population of cells wherein the female sperm cells are at least about 100% greater than the male sperm cells.

114. (Withdrawn) The method of claim 106, wherein the cell binding agent comprises a bead support having a diameter of 0.1 to 2 microns.

115. (Withdrawn) The method of claim 106, wherein the sperm cells are selected from the group consisting of cattle, sheep, pigs, goats, horses, dogs and cats.

116. (Withdrawn) A method for determining the optimum time to incubate a sample of semen prior to contacting the sample with a binding agent in order to enrich for female sperm cells, the method comprising:

- incubating a test specimen at a constant temperature for two or more different incubation periods;

- contacting the test specimen at the end of the two or more incubation periods with a binding agent which binds the selected population of cells;

- separating the test specimen into binding agent bound and binding agent unbound test specimens; and

- determining the time at which the maximum percent female cells occurs in the separated test semen specimens, wherein said time at which the maximum percent of female cells occurs is determined by plotting a curve which is obtained by plotting the percent female cells determined by FISH in the unbound test specimens against the percent of H-Y positive cells.

117. (Withdrawn) The method of claim 116, wherein the binding agent is a H-Y binding agent.

118. (Withdrawn) The method of claim 117, wherein the H-Y binding agent is a Koo antibody and the H-Y positive cells are Koo positive cells.

119. (Withdrawn) The method of claim 116, wherein the separated test semen comprises a population of cells wherein the female sperm cells are at least about 10% greater than the male sperm cells.

120. (Withdrawn) The method of claim 116, wherein the separated test semen comprises a population of cells wherein the female sperm cells are at least about 20% greater than the male sperm cells.

121. (Withdrawn) The method of claim 116, wherein the separated test semen comprises a population of cells wherein the female sperm cells are at least about 50% greater than the male sperm cells.

122. (Withdrawn) The method of claim 116, wherein the separated test semen comprises a population of cells wherein the female sperm cells are at least about 100% greater than the male sperm cells.

123. (Withdrawn) The method of claim 116, wherein the cell binding agent comprises a bead support having a diameter of 0.1 to 2 microns.

124. (Withdrawn) The method of claim 116, wherein the sperm cells are selected from the group consisting of cattle, sheep, pigs, goats, horses, dogs and cats.

125. (Withdrawn) A method for determining the optimum time at which to separate male and female sperm cells, the method comprising:

- (a) collecting a test specimen of semen;
- (b) cooling the test specimen to a predetermined temperature;
- (c) incubating the test specimen at the predetermined temperature for two or more different incubation periods; and
- (d) determining the percent of sperm cells exhibiting sticky patches in the samples at the two or more different incubation periods, wherein the optimal time for

separating male and female sperm cells is the incubation period which resulted in at least about 20% of the sperm cells exhibiting sticky patches.

126. (Withdrawn) The method of claim 125, wherein the percent of sperm cells exhibiting sticky patches is determined by labeling with Koo antibody.

127. (Withdrawn) The method of claim 125, wherein the optimal time for separating male and female sperm cells is the incubation period which resulted in at least about 25% of the sperm cells exhibiting sticky patches.

128. (Withdrawn) The method of claim 125, wherein the optimal time for separating male and female sperm cells is the incubation period which resulted in at least about 30% of the sperm cells exhibiting sticky patches.

129. (Withdrawn) The method of claim 125, wherein the optimal time for separating male and female sperm cells is the incubation period which resulted in at least about 20% but no more than 40% of the sperm cells exhibiting sticky patches.

130. (Withdrawn) The method of claim 125, further comprising separating the test specimen into biding agent bound and binding agent unbound test specimens at the optimal time.

131. (Withdrawn) A method for separating a selected population of cells from a sample of semen, the method comprising:

- cooling the semen to a predetermined temperature;

- incubating the semen for a predetermined amount of time at the predetermined temperature, wherein the predetermined amount of time was determined by a method comprising the steps of:

- collecting a test specimen of semen;

- incubating the test specimen at a constant temperature for two or more different incubation periods;

contacting the test specimen at the end of the two or more incubation periods with a binding agent which binds the selected population of cells,

separating the test specimen into selected and non selected test specimens, and

determining which of the two or more incubation time periods yielded the maximum percent female cells in the non selected test specimens, thereby identifying the predetermined amount of time for incubating the semen;

contacting the sample with a cell binding agent to preferentially bind to the selected population of cells for a time sufficient for the cell binding agent to bind the selected cells; and

separating the selected cells and the cell binding agent to provide a treated sample containing non selected cells.

132. (Withdrawn) The method of claim 131, wherein the treated sample comprises cells exhibiting a preferred sex type that is at least about 10% greater than the number of cells exhibiting a non preferred sex type.

133. (Withdrawn) The method of claim 131, wherein the treated sample comprises cells exhibiting a preferred sex type that is at least about 20% greater than the number of cells exhibiting a non preferred sex type.

134. (Withdrawn) The method of claim 131, wherein the treated sample comprises cells exhibiting a preferred sex type that is at least about 50% greater than the number of exhibiting a non preferred sex type.

135. (Withdrawn) The method of claim 131, wherein the treated sample comprises cells exhibiting a preferred sex type that is at least about 100% greater than the number of exhibiting a non preferred sex type.

136. (Withdrawn) The method of claim 131, wherein the cell binding agent comprises a bead support having a diameter of 0.1 to 2 microns.

137. (Withdrawn) The method of claim 131, wherein the number of cells in the selected population of cells is greater than about  $1 \times 10^5$  cells/ml.

138. (Withdrawn) A method of insemination, the method comprising obtaining semen containing a population of spermatozoa according to the method of claim 131 and inseminating a mammal with the treated sample containing a population of spermatozoa.

139. (Withdrawn) The method of claim 138, wherein the mammal is selected from the group consisting of cattle, sheep, pigs, goats, horses, dogs and cats.

140. (Withdrawn) The method of claim 138, wherein the semen is cooled to at least about 16°C.

141. (Withdrawn) The method of claim 138, wherein the semen is cooled to at least about 12°C.